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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: TAKESHI FUJITA ET AL.

Divisional of
Serial No.: 08/552,496 Prior Group Art Unit: 1653

Filed: May 3, 2000 Prior Examiner: J. Taylor

Title: DNA ANALYZING METHOD AND DEVICE THEREFOR

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please enter the following amendments to the Specification and the Claims prior to the examination of the divisional application.

In the Substitute Specification:

Page 3, line 17, change "ln" to --in--.

Page 7, line 11, change "hysterels" to --hysteresis--.
line 17, change "sift" to --shift--.

Page 9, lines 11 and 17, change "QA1" (each occurrence) to --DQA1--.

Page 10, line 6, change "hysteresls" to -hysteresis-.

Page 11, lines 19 and 23, change "regulator" (each occurrence) to --controller--.

Page 13, line 10, change "PCR" to --polymerase chain reaction (PCR)--.

Page 20, line 25, change "ln" to --in--.

Page 24, line 22, change "device" to --detector--.

Page 25, line 19, change "ln" to --in--.

Page 28, lines 15-16 and 26, change "regulator" (each occurrence) to --controller--.

Page 30, line 1, change "regulator" to --controller--.

In the Claims:

Please cancel claims 1-46 without prejudice or disclaimer, and substitute therefor new claims 47-61 as follows:

--47. A DNA analyzing method comprising:

preparing a sample single-stranded DNA fragment from a sample double-stranded DNA fragment,

denaturing the conformation of the sample single-stranded DNA fragment under a given denaturing condition,

measuring a melting curve of the conformation of the sample single-stranded DNA fragment to derive melting curve data representing a relation between the given denaturing condition and an obtained denaturing result, and

comparing the measured melting curve data of the sample single-stranded DNA fragment with known melting

curve data preliminarily prepared by using single-stranded DNA fragments of a known sequence for obtaining sequence information of the sample single-stranded DNA fragment and for analyzing for a DNA polymorphism including a single-base substitution in the sample single-stranded DNA fragment.

48. The DNA analyzing method of claim 47, wherein the conformation of the sample single-stranded DNA fragment is thermally denatured and the melting curve data is derived from a change of an ultra violet absorbance.

49. The DNA analyzing method of claim 47, further comprising:

alternating changing the denaturing conditions so as to denature and then renature the conformation of the sample single-stranded DNA fragment in a continuous manner, and

measuring and analyzing hysteresis characteristics of the change in absorbance.

50. A DNA analyzing method comprising:

preparing a sample single-stranded DNA fragment from a sample double-stranded DNA fragment in a sample solution,

intercalating an intercalater capable of emitting fluorescence of a given wavelength upon receiving an excitation beam of another given wavelength when it intercalates into the base pairing nucleotides formed by a conformation of the sample single-stranded DNA fragment in the sample solution, wherein a wavelength

of the fluorescence emitted from a complex of the intercalater and the sample single-stranded DNA fragment is different from the wavelength emitted from the intercalater per se,

irradiating the excitation beam of the given wavelength onto the complex of the intercalater and the sample single-stranded DNA fragment,

denaturing the conformation of the sample single-stranded DNA fragment in the sample solution under a given denaturing condition while irradiating the excitation beam,

detecting the change in an intensity of the fluorescence of a preset wavelength due to the denaturation of the sample single-stranded DNA fragment,

measuring a melting curve of the conformation of the sample single-stranded DNA fragment to derive melting curve data representing a relation between the given denaturing condition and an obtained denaturing result, and

comparing the measured melting curve data of the sample single-stranded DNA fragment with known melting curve data preliminarily prepared using single-stranded DNA fragments of a known sequence for obtaining sequence information of the sample single-stranded DNA fragment and for analyzing for a DNA polymorphism including a single-base substitution in the sample single-stranded DNA fragment.

51. The DNA analyzing method of claim 50, wherein the comparison of the measured melting curve data of the sample single-stranded DNA fragment with the known melting curve data comprises,

comparing the measured melting curve data of the sample single-stranded DNA fragment with a data set of the known melting curves or with a data set of curves prepared by linear combination of a plurality of known curve data sets, and

determining that the data set of the known melting curve with a least statistical error or the linear combination of the data sets with a least statistical error represents the sequence information of the sample single-stranded DNA fragment.

52. The DNA analyzing method of claim 50, wherein the comparison of the measured melting curve data of the sample single-stranded DNA fragment with the known melting curve data comprises,

calculating a statistical error between the measured melting curve data and a data set of the known melting curves or a data set of the curves prepared by linear combination of a plurality of known melting curve data sets, thereby selecting one curve data with a least statistical error for carrying out the calculation and selection over a remaining data set of the known melting curves or a data set of the curves prepared by linear combination of a plurality of the known melting curves, and

representing a given number of the curve data sets in the increasing order of the least statistical error as the sequence information of the measured sample single-stranded DNA fragment.

53. The DNA analyzing method of claim 50, wherein the conformation of the sample single-stranded DNA fragment is thermally denatured and the melting curve data is derived from

a change of an ultraviolet absorbance or the intensity of the fluorescence of the sample versus a change of temperature.

54. The DNA analyzing method of claim 50, further comprising:

alternating changing the denaturing conditions so as to denature and then renature the conformation of the sample single-stranded DNA fragment in a continuous manner, and

measuring and analyzing hysteresis characteristics of the change in fluorescence intensity.

55. The DNA analyzing method of claim 51, further comprising:

alternating changing the denaturing conditions so as to denature and then renature the conformation of the sample single-stranded DNA fragment in a continuous manner, and

measuring and analyzing hysteresis characteristics of the change in fluorescence intensity.

56. The DNA analyzing method of claim 52, further comprising:

alternating changing the denaturing conditions so as to denature and then renature the conformation of the sample single-stranded DNA fragment in a continuous manner, and

measuring and analyzing hysteresis characteristics of the change in fluorescence intensity.

57. The DNA analyzing method of claim 53, further comprising:

alternating changing the denaturing conditions so as to denature and then renature the conformation of the sample single-stranded DNA fragment in a continuous manner, and

measuring and analyzing hysteresis characteristics of the change in absorbance or fluorescence intensity.

58. The DNA analyzing method of claim 50, wherein the intercalater is ethidium bromide.

59. The DNA analyzing method according to Claim 50, wherein the comparison of the measured melting curve data of the sample single-stranded DNA fragment with the known melting curve data comprises,

comparing the measured melting curve data of the sample single-stranded DNA fragment with a linear combination of the known melting curve data each preliminarily fitted by a superposition of given functions, and

determining that a known melting curve data set with a least statistical error is defined as the sequence information of the sample single-stranded DNA fragment.

60. A DNA analyzer comprising:

an amplification reaction cell in which a membrane immobilizing an amplification reaction primer is placed, and in which a selective amplification of a specific DNA region is carried out and sample single-stranded DNA fragments as an analytical subject are produced;

a spectroscopic cell in which a sample solution containing the sample single-stranded DNA fragments produced in the amplification reaction cell is held;

a transferring means which transfers the sample solution containing the sample single-stranded DNA fragments from the amplification reaction cell into the spectroscopic cell;

a spectroscopic means for measuring an ultraviolet absorbance of the sample single-stranded DNA fragments in the sample solution;

a denaturing means for denaturing the conformation formed by the sample single-stranded DNA fragments in the sample solution under a preset denaturing condition; and

a signal processing device which presets the denaturing condition and saves signals from said spectroscopic means and said denaturing means, and which prepares the measured melting curve data of the sample single-stranded DNA fragments based on the saved signals, and subsequently compares the measured melting curve data of the sample single-stranded DNA fragments with known melting curve data preliminarily prepared by using single-stranded DNA fragments of a known sequence for obtaining a sequence information of the sample single-stranded DNA fragment and for analyzing for a DNA polymorphism including a single-base substitution in the sample single-stranded DNA fragment,

wherein said DNA analyzer continuously carries out a flow system from DNA amplification reaction as a preliminary treatment to the melting curve measurement.

61. The DNA analyzer of claim 60, wherein a plurality of amplification reaction cells are attached to the spectroscopic cell through valves, and the sample solution containing the sample single-stranded DNA fragments produced in each of the amplification reaction cells being introduced sequentially into the spectroscopic cell.--

REMARKS

The above amendments have been made to correct mistakes found in the substitute Specification and to put the claims into better form for consideration under U.S. practice.

Favorable action on the application is earnestly solicited.

Respectfully submitted,

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